

Sustainable Management Tactics for Control of *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae) on Canola in Montana

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ABSTRACT The crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae), has recently emerged as a serious pest of canola (*Brassica napus* L.) in Montana. The adult beetles feed on canola leaves, causing many small holes that stunt growth and reduce yield. In 2013, damage to canola seedlings was high ($\approx 80\%$) in many parts of Montana, evidence that when flea beetles emerge in large numbers, they can quickly destroy a young canola crop. In the current study, the effectiveness of several biopesticides was evaluated and compared with two insecticides (deltamethrin and bifenthrin) commonly used as foliar sprays as well as seed treatment with an imidacloprid insecticide for the control of *P. cruciferae* under field conditions in 2013. The biopesticides used included an entomopathogenic nematode (*Steinernema carpocapsae*), two entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium brunneum*), neem, and petroleum spray oils. The control agents were delivered in combination or alone in a single or repeated applications at different times. The plant-derived compound neem (azadirachtin), petroleum spray oil, and fatty acids (M-Pede) only showed moderate effect, although they significantly reduced leaf injuries caused by *P. cruciferae* and resulted in higher canola yield than the untreated control. Combined use of *B. bassiana* and *M. brunneum* in two repeated applications and bifenthrin in five applications were most effective in reducing feeding injuries and improving yield levels at both trial locations. This indicates that entomopathogenic fungi are effective against *P. cruciferae*, and may serve as alternatives to conventional insecticides or seed treatments in managing this pest.

KEY WORDS *Phyllotreta cruciferae*, *Brassica napus*, entomopathogen, biopesticide

The crucifer flea beetle, *Phyllotreta cruciferae* (Goeze), and the striped flea beetle, *Phyllotreta striolata* (F.) (Coleoptera: Chrysomelidae), are the two most serious insect pests of canola (*Brassica napus* L. and *Brassica rapa* L.; Gavloski et al. 2011). These two flea beetles share a common host range and feed on various species of Brassicaceae, Polygonaceae, Chenopodiaceae, Boraginaceae, and Asteraceae (Burgess 1977). Although both species were introduced from Eurasia and are known to attack canola, *P. cruciferae* is the only flea beetle pest of oilseed *Brassica* (canola) found in the Golden Triangle area of Montana (An area of Montana known for good wheat-growing conditions is nicknamed “The Golden Triangle.” The three points of the triangle in north-central Montana are Havre, Conrad, and Great Falls), possibly because as Tansey et al. (2008) reported, *P. cruciferae* is likely a more effective competitor than *P. striolata* under most field conditions. Adult flea beetles emerge in the spring and feed on cotyledons and true leaves (Parker et al. 2012). When in large numbers, they can quickly devastate a seedling canola field, making the timely

detection and management of this pest important (Brandt and Lamb 1993).

According to Westdal and Romanow (1972), *P. cruciferae* has a single generation per year in the northern Great Plains and is usually univoltine in North America. The species overwinters as adults in the leaf litter of shelterbelts or grassy areas, and beetles are rarely found in canola stubble (Ulmer and Dossdall 2006). Beetles emerge when temperatures approach 14°C (57 °F) in early spring, feed on volunteer canola and weeds, such as wild mustard, and then move into newly sown canola as plants emerge (Soroka and Elliott 2011). Depending on temperature, it may take up to 3 wk for all adults to leave their overwintering sites (Westdal and Romanow 1972). Warm, dry, and calm weather promotes flea beetle flight and feeding throughout the field, while simultaneously slowing canola growth (Turnock 1987). In contrast, cool, rainy, and windy conditions reduce flight activity, and flea beetles walk or hop, leading to concentrations of beetles and injury along field margins (Lamb 1989).

Canola seedlings can compensate for defoliation levels up to 20% (Gavloski and Lamb 2000, Bartelt et al. 2001). Neonicotinoid insecticides, applied ei-

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Table 1. Materials and rates used in the field trials

Material	Active ingredient	Rate	Source
Gaucho	Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine 48.7% and other ingredient 51.3%	190 ml/45 kg of seed	Bayer Crop Science, Durham, NC
<i>Azadirachta indica</i> (Aza-Direct) spray	Azadirachtin 1.2%, other ingredients 98.8%	10 ml/liter water	Gowan Company, Yuma, AZ
Millenium (Nematode)	<i>Steinernema carpocapsae</i> paste (active)	1 ml/13 liters	Becker Underwood, Ames, IA
Petroleum spray oil (Volck oil spray)	Petroleum oil 97%, other ingredients 3%	20 ml/liter	The Ortho Group, Marysville, OH
Botanigard 22WP	<i>Beauveria bassiana</i> Strain GHA 22%, inert ingredients 78%	2.4 g/liter	Laverlam International Corporation, Butte, MT
Met 52 G	<i>Metarhizium brunneum</i> Strain F52	5 g/liter	Novozymes, Davis, CA
M-Pede (GF-1212)	Potassium salts of fatty acids 49% and propylene glycol 37.4% and other ingredient 13.6%	62.5 ml/liter	Gowan Company, Yuma, AZ
Delta Gard G	Deltamethrin 4.75%	9.7 g/m ²	Bayer Crop Science, Durham, NC
Hero	Bifenthrin 11.25%, zeta-cypermethrin 3.75%, and other ingredients 85.00%	2.5 ml/liter	FMC Corporation, Philadelphia, PA

ther as seed treatment or foliar sprays, are currently used as the main approach for the control of *P. cruciferae*. Often, insecticidal seed treatment with imidacloprid is used throughout the northern Great Plains of North America to systemically protect canola seedlings from flea beetle attack (Tansey et al. 2008). Meanwhile, foliar spray of insecticides, i.e., deltamethrin or bifenthrin, is also used to prevent *P. cruciferae* from causing further significant feeding damage. To protect the crop from yield losses, these insecticides are often applied at an economic threshold of 25% feeding injury to cotyledons and first true leaves (Weiss et al. 1991). However, owing to environmental concerns (i.e., impact on pollinators and natural enemies) and pest resurgence arising from the repeated and heavy use of chemical insecticides, more environmentally compatible tactics are desirable for sustainable management of this pest.

Under these circumstances, biorational insecticides, i.e., entomopathogenic nematodes, fungi, and plant extracts, are explored for their potential as alternatives to conventional insecticides to be incorporated into an integrated pest management (IPM) program. Entomopathogenic nematodes and fungi appear to be environmentally safe and IPM compatible alternatives to conventional insecticides. In particular, pathogenicity has been reported on a fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. infecting *P. cruciferae*, although varying with fungal strains (Miranpuri and Khachatourians 1995). However, there are few reports on testing the field efficacy of these entomopathogens against *P. cruciferae*. Furthermore, biopesticides neem (azadirachtin; Antwi et al. 2007, Boopath et al. 2010) and fatty acids (M-Pede; Natwick et al. 2009) have been reported to reduce feeding injuries of *P. cruciferae*.

In the current study, we evaluated the field efficacy of an entomopathogenic nematode, two fungi, botanical insecticides, and reduced-risk insecticides used as a single or repeated applications at different times against *P. cruciferae*.

Materials and Methods

Trial Location. Trials were conducted at two field locations: Cut Bank (48° 50.292' N, 112° 17.746' W) and Sweet Grass (48° 57.831' N, 111° 40.801' W) in the Golden Triangle area of Montana. Experiments were carried out from May–September 2013 at Cut Bank and from June–September 2013 at Sweet Grass. Hy-Class 955 canola seeds were used for both locations, and the crop was seeded at a rate of 12 seeds per 30 cm using a four-row plot drill. At both locations, rows within the plots were spaced 0.3 m apart, and the herbicide Roundup Powermax (Glyphosate) at the rate of 2.5 liters/ha was applied before planting for weed control. Fertilizer N, P, K, and S ratio was applied at 134.5, 25.2, 61.6, and 22.4 kg/ha at actual time of planting and an additional application of 12.3, 25.2, and 0 kg/ha was broadcast through the seed plot drill. No irrigation was used, as the trials were conducted under dry conditions.

Materials and Treatments. Materials used in field trials include an entomopathogenic nematode (*Steinernema carpocapsae*), two fungi (*B. bassiana* and *Metarhizium brunneum*), petroleum spray oil (PSO), neem (azadirachtin), fatty acids (M-Pede), two chemical insecticides (deltamethrin and bifenthrin) currently being used by growers as foliar sprays, and an imidacloprid (Gaucho) often used as a standard seed treatment. The source and rate of materials used are listed in Table 1. Treatment with control agents was made as single or repeated applications at different days after sowing (DAS).

At each trial location, there were 12 treatments, including an untreated control (no spray or seed treatment); seed treatment with imidacloprid (no spray); two applications of *S. carpocapsae* made at 15 and 30 DAS; four applications of *S. carpocapsae* made at 15, 30, 45, and 60 DAS; one application of PSO at 15 DAS + one application of neem at 30 DAS; two applications of PSO at 15 and 30 DAS + two applications of neem at 45 and 60 DAS; one application of *B. bassiana* at 15 DAS + one application of *M. brunneum* at 30 DAS; two applications of *B. bassiana* at 15 and 30 DAS + two

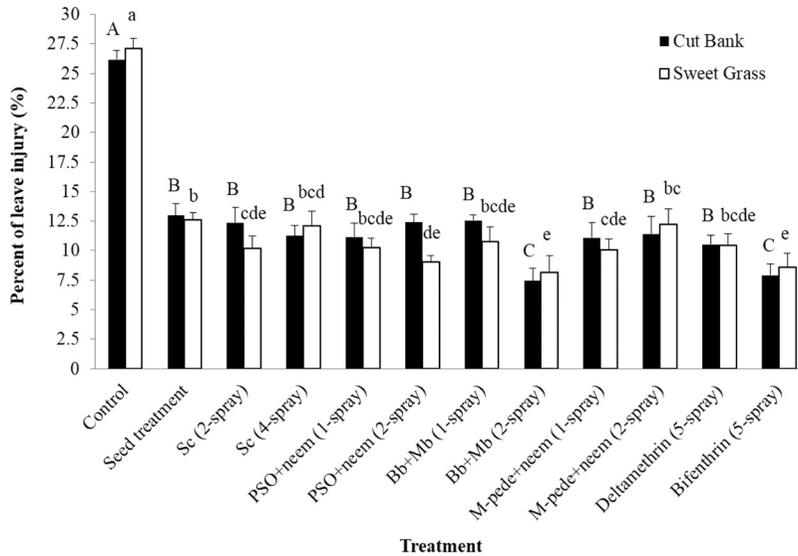


Fig. 1. Percent of leaf injury caused by *P. cruciferae* in various treatments at two field locations (Cut Bank and Sweet Grass) in Montana (mean \pm SE). Different capital and lower-case letters above the bars indicate significant differences among treatments in Cut Bank (one-way ANOVA: $F = 9.51$; $df = 11$; $P < 0.0001$) and Sweet Grass (one-way ANOVA: $F = 35.14$; $df = 11$; $P < 0.0001$), respectively (LSD test, $\alpha = 0.05$). Sc, *S. carpocapsae*; PSO, petroleum spray oil; Bb, *B. bassiana*; Mb, *M. brunneum*.

applications of *M. brunneum* at 45 and 60 DAS; one application of M-Pede at 15 DAS + one application of neem at 30 DAS; two applications of M-Pede at 15 and 30 DAS + two applications of neem at 45 and 60 DAS; five applications of deltamethrin at 10, 20, 30, 40, and 50 DAS; and five applications of bifenthrin at 10, 20, 30, 40, and 50 DAS. Seeds were not treated, except for those used in the standard seed treatment plots. Treatment plots were arranged in a randomized complete block design with three replicates.

Plot Design and Data Collection. Treatments were applied in plots measured 8 by 4 m, and were separated from each other by 1-m buffer zones to avoid contamination from spray drift. Each plot consisted of 12 rows of 80 canola plants, for a total of 960 plants per plot.

Feeding injuries and yield levels in each treatment plots were evaluated to compare the effectiveness of treatments. To assess feeding injuries caused by *P. cruciferae*, 1-m² area was randomly chosen in each plot, and all plants and leaves within the chosen area were sampled every week. The amount of leaf area injured by *P. cruciferae* was measured and compared with the total leaf surface area to calculate the percentage of leaf injury on each leaf. The number of *P. cruciferae* per plant could not be measured, as beetles are highly mobile.

The crop was harvested when $\approx 50\%$ of the seed in the pods about half way up the main stem turned dark. Plots were allowed to air dry and the seed to ripen completely ≈ 7 –10 d. A Hege 140 plot combine was used to thrash the canola plots to collect seeds for yield evaluation.

Statistical Analyses. Data on feeding injury and yield levels within treatments at two trial locations

were analyzed using a two-way ANOVA. Differences between treatments at each location were tested using Fisher Least Significant Difference (LSD) Test (one-way ANOVA). Difference was considered significant when $P \leq 0.05$. Data were analyzed using PROC GLIMMIX in SAS version for Windows (SAS Institute 2009).

Results

In both trials, all treatments (entomopathogenic nematodes and fungi, botanical insecticide, and insecticides) significantly reduced the percentage of leaf injury compared with the untreated control ($F = 30.41$; $df = 11$; $P < 0.0001$; Fig. 1). There was no significant difference in feeding injuries between the two locations ($F = 1.81$; $df = 1$; $P = 0.1856$); no significant interaction was found between treatment and location ($F = 0.39$; $df = 11$; $P = 0.9520$; two-way ANOVA). Among treatments, the combined use of *B. bassiana* and *M. brunneum* in two repeated applications and treatment with five applications of bifenthrin had significantly lower damage compared with other treatments at both locations ($P < 0.05$; Fig. 1).

Significant difference in yield production was detected at both trial locations ($F = 10.16$; $df = 11$; $P < 0.0001$). Yield produced from the two locations differed significantly from each other ($F = 338.50$; $df = 1$; $P < 0.0001$). Significant interaction was detected between treatment and trial location ($F = 5.41$; $df = 11$; $P = 0.0003$; two-way ANOVA). At Cut Bank location, the seed treatment with an imidacloprid insecticide, the combined use of *B. bassiana* and *M. brunneum* in two repeated applications, and five applications of bifenthrin significantly improved the

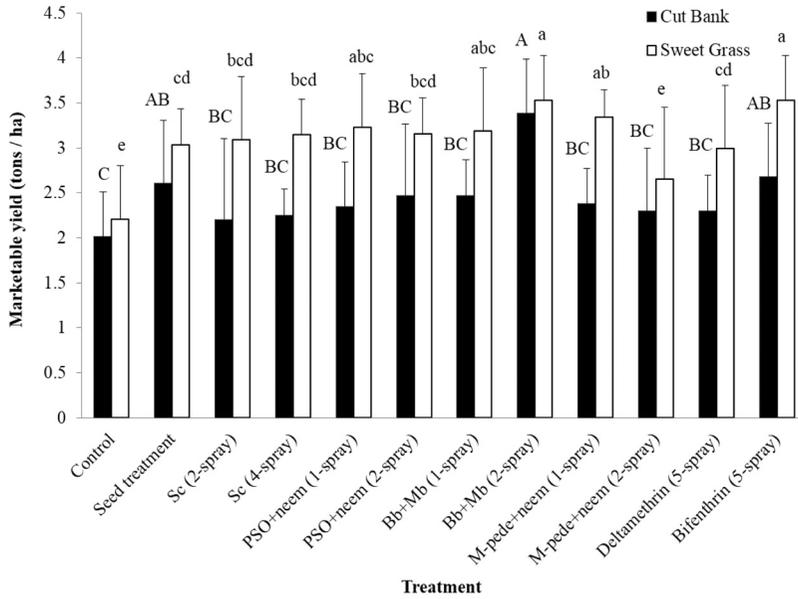


Fig. 2. Yield of canola in various treatments against *P. cruciferae* at two field locations (Cut Bank and Sweet Grass) in Montana (mean \pm SE). Different capital and lower-case letters above the bars indicate significant differences among treatments in Cut Bank (one-way ANOVA: $F = 7.17$; d.f. = 11; $P = 0.0010$) and Sweet Grass (one-way ANOVA: $F = 8.59$; d.f. = 11; $P = 0.0004$), respectively (LSD test, $\alpha = 0.05$). Sc, *S. carpocapsae*; PSO, petroleum spray oil; Bb, *B. bassiana*; Mb, *M. brunneum*.

marketable yield production (from 2 tons/ha to up to 3.4 tons/ha; $P < 0.05$). At Sweet Grass location, most treatments significantly improved the yield level (from 2.2 tons/ha to up to 3.5 tons/ha; $P < 0.05$), except the treatment with two applications of M-Pede at 15 and 30 DAS plus two applications of neem at 45 and 60 DAS (Fig. 2).

Being consistent with injury levels, the canola yield was significantly higher in plots treated with both *B. bassiana* and *M. brunneum* in two repeated applications and in plots treated with five applications of bifenthrin compared with most other treatments ($P < 0.05$; Fig. 2). In particular, two applications of *B. bassiana* and *M. brunneum* resulted in higher yield than bifenthrin at Cut Bank, although not significantly different. This indicates that the combined use of the two fungi may achieve an effect comparable or superior to the insecticide.

Discussion

In canola cropping systems, predators and parasitoids provide only limited control of *P. cruciferae* (Wylie 1984), and canola growers depend on insecticidal treatments applied to seeds or foliage. Canola growers in Montana often consider synthetic pyrethroids such as deltamethrin or bifenthrin to be the only effective method of managing *P. cruciferae*, despite their toxicity to natural enemy insects (Desneux et al. 2007) and pollinators (Bernal et al. 2010, Mommaerts and Smaghe 2011). Although there are many synthetic pyrethroids and carbamates labeled for control of flea beetle in *Brassica* crops, their repeated use

over multiple pest generations in the same location is likely to lead to the development of pesticide resistance (Turnock 1994).

In our study, we estimated the damage level in different treatment plots to evaluate the effectiveness of various treatments. We could not sample the *P. cruciferae* population because the adults are highly mobile and because of strong winds in the experimental areas during the study period. In a previous study by Andersen et al. (2006), researchers used yellow sticky traps or direct visual counts when estimating flea beetle populations in the field, but it was not possible to use this same method in our experiment because of the small size of the experimental plots and because of high winds there will be high immigration and emigration of the insect populations.

We found that canola seeds treated with imidacloprid (Gaucho) experienced lower levels of damage and gave higher yields than the controls. This observation is in agreement with Lenssen et al. (2007), who reported that plots whose seeds were not treated with imidacloprid suffered more damage than other plots. The use of neonicotinoids as a seed treatment to control insect pests has long been practiced by growers, but some studies have reported these chemicals to be toxic to bees (Decourtye and Devillers 2010). But Cutler and Scott-Dupree (2007) reported canola seeds treated with insecticides to have no long-term impact on honey bees. However, imidacloprid resistance has been reported in a range of other species, including field populations (Zhao et al. 1995, Grafius and Bishop 1996).

In this study, the combination of two entomopathogenic fungi (*B. bassiana* + *M. brunneum*) significantly lowered the level of damage by flea beetles, leading to higher canola yields. We believe that *P. cruciferae* is highly susceptible to the pathogens than the other treatments we have used in the field. Entomopathogenic fungi are effective at controlling many insect pests (Feng et al. 1990), and Miranpuri and Khachatourians (1995) have shown some strains of *B. bassiana* to be pathogenic to *P. cruciferae*. On other hand, *Metarhizium anisopliae* (Metchnikoff) Sorokin is known to infect >200 insect pest species (Roberts and St. Leger 2004). Its use as a biopesticide has been facilitated by methods for its mass culture and the commercial development of infective spores formulated as a dust.

In the current study, the treatment with entomopathogenic nematodes gave considerable control of *P. cruciferae*. As far as we know, there has been few studies on the use of nematodes for the control of flea beetles. A good example is the study of Oldham (1933, 1935) who reported the parasitic nematode *Howardula phyllotreta* Oldham causing infection in the body cavities of flea beetles. In an earlier study of Morris (1987), single treatments with *Steinernema feltiae* Filipjev before or after colonization did not provide control of *P. cruciferae* in caged microplots, owing to decline in the nematode infectivity.

The treatment with petroleum spray oils or volck oil (PSOs, or horticultural mineral oils) likewise was proven to be effective in reducing flea beetle damage levels and increasing crop yield compared with the control (Feeny et al. 1970). Because of their low toxicity to natural enemies and safety to the environment, PSOs are IPM-compatible pest control compounds (Beattie and Smith 1996). However, there has been no practical work done using PSO for the control of flea beetles. PSOs, because of their short-term residual activity, do not severely affect populations of beneficial arthropods, although predators and parasites may be killed on contact when sprayed directly (Davidson et al. 1991).

In our study, the treatment with neem also had some effect in controlling *P. cruciferae*. Similar field studies in India have found a 100% reduction of *P. cruciferae* by the seventh day after application (Boopath et al. 2010), and neem extracts have been reported to be an effective treatment against *P. cruciferae* (Antwi et al. 2007) and *P. striolata* (Hou et al. 2003). Neem biopesticides may, therefore, be well suited for inclusion in IPM programs (Schmutterer 1990, Immaraju 1998, Reddy and Guerrero 2000). Similarly, M-Pede was more or less as effective as the neem treatment. Natwick et al. (2009) reported that M-Pede provided partial control of flea beetles when plants were in the cotyledon stage. Although M-Pede is commercially available, there are few studies on its effectiveness against various insect pests and natural enemies.

To conclude, the combined use of *B. bassiana* and *M. brunneum* in two repeated applications and in five applications were most effective in reducing feeding injuries and improving yield levels at both trial loca-

tions. The combination of entomopathogenic fungi achieved an effect comparable or superior to insecticides, and has potential to be used as an ecologically friendly alternative to conventional insecticides or seed treatments for the control of *P. cruciferae*. Further research on the development of sustainable pest management programs based on threshold-based monitoring may advance the strategies for managing *P. cruciferae*.

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